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A BACILLUS FROM SPONTANEOUS ABSCESES IN RABBITS AND ITS RELATION TO THE INFLUENZA BACILLUS.*

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For several years past, and especially during the last year, there have come under my observation many rabbits with abscesses with certain rather characteristic features. Interest in these abscesses was aroused because a pleomorphic bacillus having some features in common with organisms of the influenza group was found in the pus, usually pure in all cases. Inasmuch as pathogenic hemophilic bacilli have never been found in animals other than man, it was deemed important to study this bacillus somewhat minutely.

The abscesses occur on the abdomen, sides, extremities, neck, and especially on the back and at the base of the ears. They may be single but often are multiple, there being at times six or eight distinct abscesses in a cluster. They always occur in the loose subcutaneous tissue and do not invade the subjacent structures. They are at first free from the skin, but later they may break down and discharge their contents. They are distinctly encapsulated and vary in size from that of a pea to that of an orange or larger. If incised and evacuated they usually disappear. The animals at first appear healthy but later usually show some emaciation which may become extreme if the abscesses grow to large size. The capsule or wall of the abscess is usually 1-3 mm. thick and consists of connective tissue which is easily ruptured. The content early is highly purulent and quite moist; later it becomes yellowish gray, dry, and cheesy in appearance. It may have a rancid, disagreeable odor. Stained smears reveal leukocytes in all stages of disintegration, much granular fatty detritus and bacteria.

Bacteriological examination.—Abscesses from 18 animals have been examined and in each there was found both in smear and culture a characteristic bacillus. As a rule it was in pure culture but in some abscesses which had previously ruptured staphylococci were mixed with it. In smears made directly from the pus the bacillus is usually rather long, irregular, often arranged in pairs end to end and sometimes in longer chains of four, six, or eight elements. The ends are rounded and at times in older pus the organism may be thread-like and tortuous. It is nearly always found outside the leukocytes. In cultures it often loses its tendency to form long threads, at least to a considerable extent. Consequently it appears usually as a small bacillus with here and there filaments of different lengths. Some strains are very small, being

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about the size of influenza bacilli but more coccoid. The thread-like filaments also resemble closely those seen in cultures of the influenza bacillus. While the size varies greatly, the average length may be given as $1-1.2 \mu$ and the width as $0.3-0.4 \mu$.

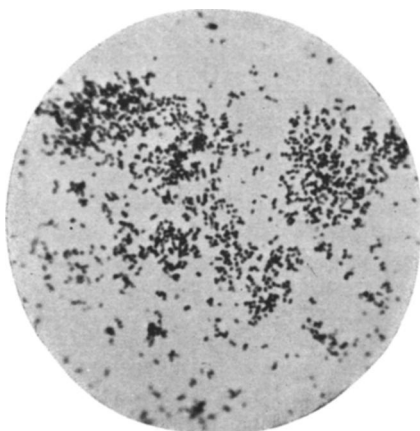


FIG. 1.—Pure culture of bacillus isolated from an abscess in a rabbit. Little or no tendency to form threads. The bacilli are about the size of the influenza bacillus. $\times 1,000$.



FIG. 2.—Pure culture of same strain as shown in Fig. 1, after growing for some time on artificial media. $\times 1,000$.

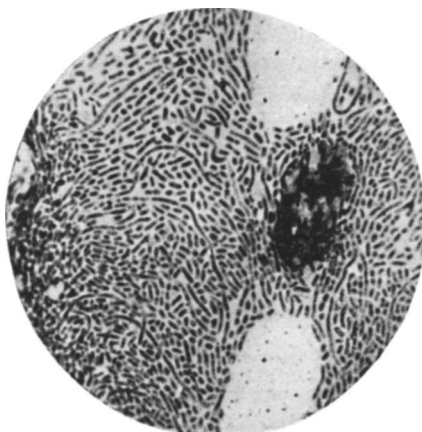


FIG. 3.—Pure culture of abscess bacillus, showing marked tendency to form filaments. A disintegrating leukocyte is seen to the right. $\times 1,000$.

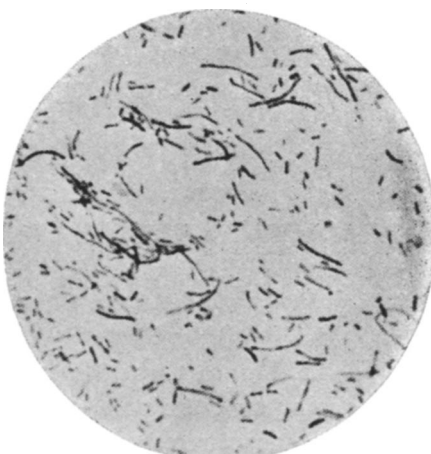


FIG. 4.—Pure culture of so-called pseudoinfluenza bacillus, a strictly hemophilic organism. Note similarity to organism shown in Fig. 2. $\times 1,200$.

In the hanging drop no definite grouping is apparent beyond that mentioned above. There is no motility, and spores and capsules have not been noted. It stains readily with ordinary dyes, the ends, as a rule, taking the stain more intensely than the middle portions. Distinct granules have not been seen in the bacillus. It destains with

gram, though perhaps with somewhat more difficulty than the influenza bacillus. It is non-acid fast.

On plain agar the growth often fails altogether when the organism is first isolated. After several generations it may become more profuse. It is gray, moist, flat, slightly spreading, translucent, and distinctly viscid; no odor and no chromogenesis.

Blood agar is by far the most favorable medium. Growth is abundant in 24 hours, quite viscid, and with the characteristics noted on plain media; no hemolysis. On serum agar and Loeffler's blood serum growth is fairly abundant, though when first isolated, the bacillus may grow very feebly or fail to grow altogether. After growing on blood agar for a time the growth on serum media may be quite as profuse as on blood media. No liquefaction of the serum media.

In agar stab cultures growth occurs along the entire needle track, best at the top but with scanty surface growth. Glycerin agar yields a more profuse growth than plain agar. Gelatin stab reveals scanty growth along the needle track and without

liquefaction. On potato as a rule there is no growth or only the merest trace; no pigment. In broth there occurs moderate turbidity without pellicle and with slight sedimentation; no odor. In litmus milk no change occurs; growth is doubtful.

The individual colonies are best observed on blood-agar plates. In 24 hours they may attain the diameter of 1-2 mm. They are grayish blue, translucent, slimy, flat, and non-hemolytic. With a low power the colonies have entire margins, are finely granular and round or slightly oval in shape.

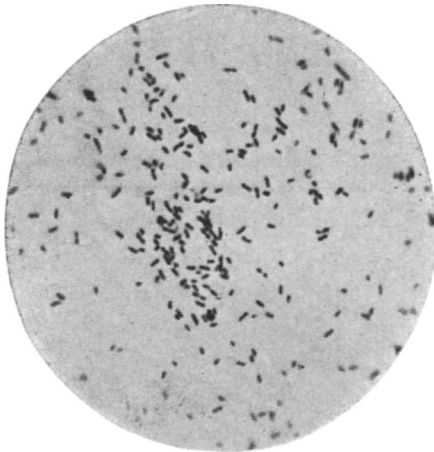


FIG. 5.—Influenza bacillus; pure culture. Compare with Fig. 1. $\times 1,200$.

Indol is not produced and gas is not formed in glucose media. The bacilli live from two to three weeks at room temperature in culture tubes

and are killed in 15 minutes at 60° C., but not at 55° C. for 30 minutes. They grow slowly at room temperature and best at 37° C.

While the observations in connection with these abscesses were going on there was noted among the animals an occasional case of pleuropneumonia. During the last year this disease became epidemic and caused the death of nearly all the animals in the laboratory at the time. This disease, known also by some as rabbit influenza, snuffles, etc., appears to be not uncommon in laboratories and probably may be caused by a number of different germs, as will be pointed out later. As regards these cases the onset was usually manifested by a profuse discharge from the nose. More

than half of the infected animals died in the course of a few days. When not fatal, recovery took place in from 10 days to two weeks. Such animals appeared to be immune to natural infection, though they were susceptible to inoculations with cultures of the bacilli. At autopsy invariably there was found a rich fibrino-purulent exudate in one or both pleural cavities and a pneumonia involving portions of a lobe or at times the entire lobe. The mucosa of the upper respiratory tract was always red and swollen. Microscopically the exudate was rich in cells. Fibrin in the alveoli and the bronchi was abundant and the parenchyma was deeply infiltrated with inflammatory cells. At times a sero-fibrinous pericarditis and also peritonitis existed. From the exudates and from the heart's blood, in every case without exception, there was isolated a small bacillus.

This bacillus is a gram negative, non-motile, polar staining minute bacillus which does not form spores and is not hemolytic. In smear preparations it is usually short and rather plump, but nearly every strain examined showed some large thread forms. In some strains they were common, in others scarce. The morphology of this bacillus together with the observation that it at times failed to grow on media not containing blood not only suggested a relation to the influenza bacillus but also suggested a possible relationship to the bacillus which had been isolated from the abscesses. A comparative study was therefore made of a number of strains isolated from animals dying of pleuro-pneumonia and strains obtained from abscesses.

Briefly, on the various culture media no essential differences were noted. Slight variations at times appeared, but only such as might occur between various strains of the same organism. On milk, potato, broth, blood agar, serum media, etc., they grew exactly alike and on various sugars they could not be differentiated. They all fermented glucose, saccharose, and mannite without gas formation and produced no change in lactose, raffinose, and maltose.

Pathogenicity.—These bacilli are invariably pathogenic for rabbits and guinea-pigs. A suspension of the bacillus from the abscesses inoculated subcutaneously into rabbits will produce lesions identical with those occurring naturally. The abscesses grow slowly and after weeks or months may attain enormous size. In the case of one animal which lived five months an oblong abscess developed at the point of inoculation

in the back, growing slowly but gradually and at the time of death measured 16 cm. in length, 8 cm. in width, and 6 cm. in depth. The animal became greatly emaciated. From the cheesy contents of the abscess the bacillus was recovered in pure culture. The heart's blood was sterile and the organs showed no important alterations. No other abscesses occurred anywhere in the body. Two rabbits were inoculated intratracheally with bacilli isolated from the abscess. One animal died a few hours later without any specific alterations in the organs. The second animal survived one week, at which time it was killed and a distinct region of bronchopneumonia nearly one centimeter across was found in the upper lobe of the right lung. In the bronchi was a small amount of purulent exudate from which the bacilli were recovered. Guinea-pigs appear to be more susceptible. Intraperitoneal injections kill in from one to three days and the organisms are found in the heart's blood and body cavities. Subcutaneously a small local abscess with edema and necrosis usually develops which may become walled off and heal or may kill by general infection. Large chronic abscesses such as occur in rabbits do not appear to develop in guinea-pigs.

The bacilli isolated from the pleuropneumonia when inoculated subcutaneously in small doses into rabbits will cause chronic abscesses in every respect like those caused by bacilli isolated from the abscesses. One drop or less of broth culture is sufficient for this purpose. If a much larger quantity is given the infection rapidly spreads in the subcutaneous tissue accompanied by intense edema and necrosis and the animal dies very soon of septicemia. In such experimental infections there may be involvement of the lungs in the form of typical pleuropneumonia, but often there is not. A number of rabbits were inoculated intravenously, and in all death ensued within 24 hours without localization. The bacilli multiplied very rapidly in the blood and appeared in large numbers in smears from the heart. The bacilli from bronchopneumonia injected into guinea-pigs subcutaneously or intraperitoneally produce lesions identical with those caused by the abscess bacillus.

Some further observations were made bearing on the pathogenicity of these bacilli and also on the possible origin of the subcutaneous abscesses. I think there is little doubt that they arise from bites and scratches received from other rabbits, though in most instances this cannot be definitely determined because the skin over the abscesses shows no ulceration or scars. The following observation was made: In the case of a large male rabbit, fatal infection resulted following a bite in the side received from another male rabbit during a fight. A large tear in the skin was found, and at this point there resulted a local acute infection with extensive edema. This infection extended along one of the large veins on the animal's side up to the subclavian vein, causing an extensive thrombosis. A generalized infection resulted in death, and from the heart's blood, peritoneum, and the lesion on the side the bacillus was obtained in pure culture. It is to be noted, however, that in this animal watery discharge from the nose and pleuropneumonia were not present. As a control to this observation a large rabbit was inoculated in the side, at approximately the same point as the above rabbit was bitten, with a large dose (one broth culture) of a strain isolated originally from a typical chronic abscess. At the site of injection there appeared a few days later extensive infiltration soon followed by death of the animal. From the infiltrated region thrombosis of the veins extended to the subclavian vein and into the heart exactly as occurred in the animal that was bitten.

From the above experiments it would seem probable that the abscesses arise from bites or scratches. The rabbit which inflicted the bite, it may be stated, was normal

in every way, though other animals in the laboratory at the time were suffering with abscesses, but none with pleuropneumonia.

There can be no doubt, then, that the bacillus found in the abscesses is the specific cause of these lesions, since typical lesions can be produced with pure cultures and from such lesions the organisms can be again recovered. The bacillus found in the lesions of pleuropneumonia is undoubtedly the specific cause of this disease also. Furthermore, the bacillus of pleuropneumonia may cause typical chronic abscesses in rabbits if small doses are given. But the results of inoculation of animals with the abscess bacillus even in large doses cannot be said to reproduce, in every respect, the clinical and pathological picture of pleuropneumonia. A pneumonia was experimentally produced by intratracheal injection, but this might be expected with almost any organism. I am inclined to believe, in view of the fact that the organisms are practically identical culturally and in other respects, that the abscess bacillus is the same organism as the pneumonia bacillus but reduced in virulence. They may, however, be closely related organisms of the same group.

In order to test this point, agglutination experiments were made with the serum of animals suffering with the disease. The serums from four rabbits which had abscesses for periods of from one to four months were tested, using both autogenous bacilli and strains which had been isolated from other animals as well as strains from cases of pleuropneumonia. Not a trace of agglutination was found in any instance. Furthermore two guinea-pigs were treated with dead bacilli and after several injections no trace of agglutinin was found in the animals. The serum from animals with pleuropneumonia likewise failed absolutely to agglutinate the bacillus causing the disease.

From these results it cannot perhaps be definitely decided whether or not the bacillus from the abscesses and that causing pleuropneumonia are identical, but they certainly appear to be very similar, and probably they are one and the same organism.

Concerning the relation of these bacilli to the influenza bacillus, morphologically they are quite similar and might readily be confused. The peculiar thread formation in both is much the same

and the character of the infection of the respiratory tract in rabbits might be considered suggestive as is indicated by the fact that the disease is sometimes known as "rabbit influenza." However, these bacilli, though preferring media containing blood, are not strictly hemophilic, as are the influenza bacilli, and they show no evidence of symbiosis which is another very distinctive property of true influenza bacilli. They should not be classed therefore in the influenza group.

From a study of the literature there is some doubt whether this organism from abscesses has been previously described. Schimmelbusch and Mühsam¹ in 1896 described an organism isolated from abscesses in rabbits which in many respects agrees with the bacillus here described and probably is identical, though their somewhat incomplete description does not permit a satisfactory comparison. Their organism was a gram negative, non-motile, short bacillus with many of the cultural features of the bacillus here described and which upon reinoculation produced similar chronic abscesses. Lexer² used this organism in certain experiments designed to produce osteomyelitis in rabbits. He found that when the bacillus alone was inoculated intravenously no results were obtained; but mixed with staphylococci abscesses were produced in bone which the cocci alone would not cause. Also, he found that if the bacillus was injected three days after a staphylococcus injection, multiple joint infection would result from which lesions the bacillus could be recovered mixed with the staphylococcus or at times pure. Lexer points out the possibility of a symbiotic relationship existing between these organisms.

In the literature are reports also of a number of varieties of bacilli which have been isolated from rabbits dying of pleuropneumonia (*Brustseuche*), but though all of these bacilli resemble each other at least in certain respects, many differ to such an extent that they cannot be considered identical.

Beck³ in his discussion of pseudoinfluenza bacilli mentions a small, gram negative, non-motile bacillus, pathogenic for rabbits,

¹ *Arch. f. klin. Chir.*, 1896, 52, p. 564.

² *Ibid.*, p. 576.

³ *Handbuch der path. Micro.*, 1903, 3, p. 405.

guinea-pigs, and mice and having a marked tendency to form threads, as the cause of *Brustseuche* in rabbits, in which condition fatal bronchopneumonia and pleurisy occur. This organism is somewhat similar to the influenza bacillus or rather the pseudo-influenza bacillus. Schimmelbusch and Mühsam discuss Beck's organism and think it slightly different from the bacillus they found in the abscesses. They point out especially that Beck's organism forms threads like the influenza bacillus, whereas their organism never does. The bacillus here described often formed filaments, in this respect more closely resembling Beck's bacillus. But neither are strictly hemophilic, nor do they show the property of symbiosis; consequently they differ from the influenza bacillus in two of its most definite and characteristic properties and should not be included in the influenza group.

Laven¹ described a bacillus pathogenic for rabbits and guinea-pigs which he said had been heretofore undescribed. He isolated it from an animal dying of *Brustseuche* and also from abscesses appearing on rabbits which seem to have been quite similar to the abscesses described in this paper. It is a small gram-negative bacillus tending to grow in chains and threads, and is variable in size. According to the description given it differs in a number of important points from the bacillus I describe. For example, it is slightly hemolytic, it gives a peculiar sperm-like odor on blood agar, and does not form acid in dextrose, saccharose, and mannite. Morphologically the two closely resemble each other.

Recently Glaue² also reports finding a small bacillus as the cause of an epidemic of *Brustseuche* in rabbits. This disease was evidently quite different from the one here described. There was never any running at the nose in the affected animals which was such a striking characteristic in our epidemic. The bacillus never formed threads and was peculiar in that the growth took on a characteristic dry appearance after 48 hours, a point to which he called especial attention. This latter point was never observed in our cultures. He does not mention its relation to abscess production in rabbits. He contends that his bacillus is different from

¹ *Centralbl. f. Bakt.*, I, Orig. 1910, 54, p. 97.

² *Ibid.*, 1911, 60, p. 176.

Laven's bacillus morphologically, culturally, and in its pathogenic effects. It was not hemolytic and growth on blood agar was not more abundant than on media without blood.

Kurita¹ described a small, gram negative, polar staining bacillus which when first isolated grew only on blood or egg media, but later would grow fairly well on plain media. He isolated this bacillus from rabbits dying of septicemia and it was pathogenic for rabbits, guinea-pigs, and mice. It killed rabbits when injected intraperitoneally or intravenously by producing *Brustseuche*, and when injected subcutaneously produced abscesses in both guinea-pigs and rabbits. He believes his bacillus is different from Beck's bacillus.

None of these bacilli correspond exactly with the bacillus described in this paper nor are any two of them exactly alike. Laven has tabulated the various germs of this group which have been found in rabbits and guinea-pigs and for detailed differences reference may be made to this paper. Including his own, 14 gram negative organisms have been described. Four are motile and one of the number liquefies gelatin. Though they all produce similar pathologic lesions in the infected animals, in many other respects they are quite different. The organisms which most closely resemble my bacillus have been referred to and pointed out above. It is to be noted that with the exception of the work of Schimmelbusch and Mühsam all of the reports deal with the relation of these bacilli to lesions of the respiratory tract. The relation between the subcutaneous abscesses and the respiratory lesions apparently has not been noted and for this reason I have called particular attention to this phase of the subject.

SUMMARY.

Spontaneous subcutaneous abscesses in rabbits are caused by a definite bacillus for which all of Koch's postulates have been fulfilled. At times the bacillus produces acute fatal infections in animals following bites. Bites or scratches are probably the common mode of infection.

¹ *Centralbl. f. Bakt.*, I, Orig. 1909, 49, p. 508.